THESIS SUBMITTED

FOR THE DEGREE OF Ph.D.

OBSERVATIONS ON THE ETIOLOGY OF CORONARY ARTERY DISEASE WITH RELATION TO CHOLESTEROL METABOLISM AND MAST CELLS

by

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INTRODUCTION

Coronary Artery Disease

Numerous attempts have been made to demonstrate a causal relationship between hypercholesterolaemia and atherosclerosis, but no such relationship has been clearly shown to exist although, equally, it has not been disproved.

Evidence yielded by a frontal attack - comparison of the plasma cholesterol levels in persons with and without clinical evidence of atherosclerosis - is inconclusive.

Acute Coronary Infarction. In a well controlled experiment Mjassnikow (1924) stated that out of 26 patients, 14 who suffered from Angina Pectoris had elevated serum cholesterol, the values ranging from 190 - 440 mg%, whereas in twenty five healthy persons the serum cholesterol ranged from 120 - 170 mg%. Mjassnikow only mentioned the term "angina pectoris", so that his group of cases were presumably not suffering from coronary infarction in the acute stage; nor is it clear whether these patients had previously suffered from acute myocardial infarction. The results in normal subjects stated by him were very low compared with those of most workers, so the question of technique may arise.

Similarly clear differences have been found by others, but this is not the general experience. A considerable overlap is a more usual finding. /

Thus Davis, Stern and Lesnick (1937) studied blood lipids and cholesterol in 59 patients with angina pectoris and in 54 controls using two different analytical methods which gave concordant results. They found that there was considerable overlapping of serum cholesterol values in the two groups and that the difference was not statistically significant, although a small group (20%) of those with angina pectoris showed values well above the highest normal value. They also found high values for the free fraction of the serum cholesterol.

Their patients were not in the acute stage of coronary infarction at the time of the examination but appro imately one third of the group gave a history consistent with cardiac infarction in the past. This group of cases was not separated from the others, the results being published as a whole so that definite conclusion cannot be reached as to the relative importance of acute coronary infarction and angina pectoris. They diagnosed the incidence of infarction from the history only, and in the absence of E.C.G. confirmation, their diagnosis of infarction cannot be accepted as absolutely definite. These workers also showed the free fraction of the plasma cholesterol to be increased in angina pectoris. Similarly Steiner and Domanski (1943) using an adaptation of Bloor's (1916) method, and studying/

serum cholesterol in patients with coronary arterio-sclerosis found that these patients had serum cholesterol levels significantly higher than in normal subject. They further observed that the serum cholesterol level fluctuated in their cases of coronary arterio-sclerosis. Fifteen cases were studied of whom 13 had coronary occlusion confirmed by electrocardiography. The measurements of serum cholesterol were made about 6 weeks after the acute attack. The remaining two patients had angina pectoris without evidence of infarction. These workers examined B.M.R. and tested for Urine sugar in each patient to exclude myxoedema or diabetes as causes of hyper-cholesterolaemia. The average serum cholesterol for individuals ranged from 296 mg% - 499 mg% with a general average for the whole series of 355 mg%; the maximum cholesterol level was 499 mg% in a patient aged 72 years and the next highest was 389 mg% in one aged 69 years. Many observers, however, have now shown that higher levels of cholesterol are usually found in younger patients rather than older so that other factors may be involved in these cases. It may be that the very high results which Steiner and Domanski obtained are due to the use of Bloor's method, which usually gives higher results than more recent procedures. Their figures for normal/

controls were also high - mean 255 mg% with a maximum of 334 mg% and a minimum of 214 mg% - so that their general conclusion is valid.

That age may be a factor in the hypercholesterolaemic tendency was pointed out by Learman and White (1946), who, however, were studying patients with angina pectoris but without acute coronary infarction. However, the same observation has been reported by others in acute coronary infarction. Morrison, Hall and Chaney (1948) studied 200 cases of coronary occlusion within 48 hours after hospital admission using Sperry's digitonin method of determination. They concluded that coronary thrombosis in patients under the age of 60 is frequently associated with hypercholesterolaemia and disturbances of cholesterol metabolism, on the grounds that (a) 68% of 75 patients under 60 years of age with proved acute coronary occlusion had hypercholesterolaemia, (b) 52% of 125 cases over 60 years of age with proved acute coronary occlusion showed a cholesterol level within the normal range, only 48% showing hypercholesterolaemia. In these cases, incidentally, the average percentage of the cholesterol in the free state was 30% compared with 28% in the normal controls. Morrison et al further concluded that high plasma cholesterol values may be an etiological factor in many cases of coronary occlusion but that/

other cases occur in which it would appear that cholesterol metabolism may not be involved. A similar tendency was noted by Collen (1949) and by Geier (1949); this last author states that whereas none of 16 healthy subjects had a plasma cholesterol concentration exceeding 300 mg. per 100 ml., this level was exceeded by 43% of patients with coronary artery disease and under 55 years old, but by 16% of similar patients over 55 years old.

These results, which form a fair cross section of those reported in the literature show that hypercholesterolaemia, in the sense of a plasma cholesterol concentration distinctly above the normal maximum, does not necessarily precede coronary artery disease. However, the normal range is so wide that the figure found in a patient with coronary arterio sclerosis may well represent a personal hypercholesterolaemia - i.e. above that previously customary for the individual concerned. It is, however, striking that such tendency to hypercholesterolaemia (in the general sense) as exists is greater in the younger patients.

Histological examination of normal, atheromatous, and sclerotic arteries shows that deposition of cholesterol in the vessel walls is an important part of the disease process (Virchow, 1924), Aschoff (1924), Leary (1944). A similar/

conclusion is given by the animal experiments of Anitschkow (1933), Leary (1941), Weinhouse and Hirsch (1940), Mukhejee (1946-1947) and others. However, although it would appear that hypercholesterolaemia may aid abnormal deposition of cholesterol in vessel walls, it has not been shown to be an essential factor, and, indeed, Duff (1935) claimed to have observed that injury to the vessels seemed to precede the deposition of cholesterol.

On the whole, it appears likely, as Peters and Van Slyke (1946) suggest, that "cholester accumulates in the walls of the arteries when these are affected by degenerative processes or suffer local injuries". Such a deposition might be aided by hypercholesterolaemia or, without actual elevation of the plasma cholesterol level, by a decreased stability in solution either of the cholesterol alone or of the colloidal solutes in general (Alvarez and Neuschlosz, (1931); Eck and Desbordes (1934); Hueper (1944) etc.). It is particularly significant that Eck and Desbordes (1935) found the stability of the plasma cholesterol to decrease with advancing age; this suggests that deposition might tend to occur at a lower plasma cholesterol concentration in old than in younger persons.

Angina Pectoris. Although some of the reports considered in the preceding section certainly included data from patients without acute coronary/

infarction, the majority dealt clearly with that condition. It is, however, important to enquire whether the tendency to hypercholesterolaemia is confined to acute coronary infarction or is found in other stages of coronary disease. This may well have a considerable bearing on the question of whether disturbance of the plasma cholesterol precedes (and may therefore be a cause of) coronary artery damage or whether the latter is the earlier phenomenon.

Bachmeister and Hens (1913), from a study of 13 cases of angina pectoris with arteriosclerosis, concluded that patients with arteriosclerosis in this stage of development showed an increase in blood cholesterol. It is true that the method available for estimation of cholesterol was far from perfect at that time, as is agreed by many observers, and that Bloor's method as then used, gave very high results. Further, the authors failed to mention the etiology of the arteriosclerotic changes. Another difficulty is that these early workers used whole blood as their analytical material. Hence their observation of high blood cholesterol cannot be accepted as definitive. In the same year Weltman (1913) also reported that 11 out of 12 persons with angina pectoris and arteriosclerosis had increased blood cholesterol/

but his observations too were open to objection. Similar findings were reported by other workers. Thus Denis (1917) found that 5 out of 14 patients with angina pectoris and arteriosclerosis had values for blood cholesterol exceeding those of 20 control persons; Gorham and Mayers (1917) reported that the range of cholesterol levels in 10 patients exceeded that in 14 normal persons. Majassnikow (1924) stated that out of 16 patients, 14 suffering from angina pectoris had a high serum cholesterol. Here he only mentioned the angina pectoris group of cases as such and did not give their history. The range which he gave - 190 - 440 mg per 100 ml. serum is contrasted with his rather surprising normal range of 120 - 170 mg% which was much lower than is nowadays generally accepted. Stepp (1918) failed to demonstrate elevation of the cholesterol content of serum and his work is noteworthy because at that period, as has been mentioned, most other observers found high cholesterol levels in angina pectoris. The reason may be that Stepp selected only these cases of angina pectoris without any other obvious cause for hypercholesterolaemia. In more recent times with improved analytical methods, Andes, Kampmeir and Adams (1936) could not find a high cholesterol level in angina using an adaptation of the method of Myers and Wardell. /

Page, Kirke and Van Slyke (1936) also failed to find high concentrations of plasma cholesterol possibly again because they employed an accurate gasometric method and diagnosed the cases accurately as angina pectoris with essential hypertension. Elliot and Nuzum (1936) using, however, whole blood by a modified Bloor's method also failed to get high results such as the whole blood samples had usually given in the hands of earlier workers.

These later results strongly support the view that the plasma cholesterol level is not increased, at least regularly, but these observers failed to classify the cases according to duration, which may well be an important factor. In contrast to these wholly positive or wholly negative results, Davis, Stern, Lesnick (1937) published a comparative study of colorimetric and gravimetric determinations of blood lipids and cholesterol. Their material included 59 cases with angina pectoris, and 54 normal controls they found that there was a considerable overlapping of serum cholesterol values in the two groups and that the differences were not statistically significant, but a small group of (20%) of those with angina pectoris showed values well above the highest normal value. Evidently the position with respect to the plasma cholesterol in angina pectoris without acute coronary/

infarction is obscure. Clarification is essential if conclusions are to be reached as to the existence of hypercholesterolaemia as a regular feature of arterial degeneration as distinct from a special feature (whether cause or effect) of coronary occlusion.

Hypertension. The level of the plasma cholesterol appears to be related to vascular degenerative change in general rather than to coronary disease in particular. This is important in itself but does not decide whether the cholesterol abnormality which frequently, though not invariably, accompanies coronary disease is a precursor of or a sequel to the vascular disease.

The relation of the serum cholesterol to hypertension seems to have first been studied by Le Moine (1911), who, using a semi-quantitative colour test, found the cholesterol content of the serum abnormally high in clinical hypertension. Pribram and Klein (1924) measured the plasma cholesterol in 47 cases of hypertension, and reported that 76% of their cases showed high values. Westphal (1924-1925), Gelman (1927), Wacker and Fahrig (1932) and Medvei (1932) have reported similar results. More recently Davis, Stern, Lesnick (1937) found high cholesterol content of blood in hypertension with arterio sclerosis/

as did Steiner and Domanski (1943). On the other hand Loewenstein (1928) reported 50 cases of hypertension of which 4 were apparently nephritic and the rest were cases of essential hypertension. Of the 50, he found that only one had serum total cholesterol above 180 mg% which he considered the maximum normal value by his calorimetric method. Harris (1930) also reported normal total cholesterol levels in hypertension whilst Weinstein and Weiss (1931) found a high total cholesterol in only 5 of the 37 cases of uncomplicated essential hypertension. Alvarez and Neuschlosz observed normal total cholesterol in hypertension; they, however, studied also the ability of the serum to dissolve additional free cholesterol and found that the sera of 21 out of 25 hypertension cases appeared to be saturated with cholesterol whereas most normal sera dissolved added cholesterol.

Kirchgessner (1934) reported that most hypertensive subjects in a series of 49 showed high values for free cholesterol although the total cholesterol was normal. Burger and Mobius (1934) observed that both free and total cholesterol were normal in hypertension.

Gibbs, Buckner & Bloor (1933) found that the group of diabetics with the most advanced clerosis showed the highest total and esterified cholesterol/

contents and that in these the proportion of cholesterol in esterified form was 10-15% above the usual normal value.

Diabetes in general is associated with some degree of hypercholesterolaemia but it is noteworthy that this is apparently intensified as vascular degeneration increases. For example Page, Kirk and Van Slyke (1936) determined the plasma cholesterol by gasometric method in 16 patients, diagnosed as essential hypertension, definitely and by proper investigation; data from 2 patients with malignant hypertension were also obtained; yet they detected no regular increase either in plasma lipids generally, nor in the free and total cholesterol ratio. Similarly Peters and Man (1943) failed to observe any high cholesterol neutral fat or lipoid phosphorus level of plasma in hypertension.

Calculation from the data of Page et al (1936) yields the noteworthy result that in their series of hypertensives the free cholesterol formed, on the average, 37% of the total plasma cholesterol, a considerable increase above the normal 27%. This is a finding of importance in relation to the view, which will be developed later in this thesis, that vascular degeneration leads slowly to deficient liver function/

and therefore to an excessive proportion of free cholesterol in the plasma.

It is evident that there is no more unanimity concerning the relation of hypertension than concerning that of coronary disease to the plasma cholesterol level. Nevertheless the evidence, taken in toto suggests that hypertension tends to be associated with some degree of hypercholesterolaemia involving principally, the free cholesterol. It may be that factors such as age of the patient, duration of the hypertension etc., have been insufficiently taken into account and that they may play an important role. Their influence will be considered in the experimental part of this thesis.

Congestive Cardiac Failure. Little attention seems to have been paid to Epstein's (1936) observation that in a short series of cases of congestive heart failure the total plasma cholesterol tended to be low although the free cholesterol was either normal or actually high. Yet this is an observation of considerable importance, for it has repeatedly been observed that in this condition the liver is frequently enlarged and that liver function tests sometimes at least give abnormal results. The findings in relation to hepatic function were well reviewed by Cantarrow (1935) and later work has emphasised the tendency to/

disturbed liver function during acute congestive heart failure with some recovery of function when the cardiac condition improves. It is obvious that further observations of the plasma cholesterol in this condition are required and, if considered in relation to the liver functional efficiency and to vascular degeneration, may provide useful information.

EXPERIMENTAL PART

METHODS

Total cholesterol was determined in plasma separated from oxalated venous blood withdrawn (in the case of the hospital patients) at 10 - 11 a.m., about three hours after breakfast. The method used was that of Sackett (1925) adapted for use with a Spekker absorptiometer.

Free cholesterol was determined in the same plasma samples by Clark's (1945) modification of the digitonin precipitation method described by Sperry (1936).

This combination of methods was chosen as being more convenient than using the digitonin method before and after hydrolysis. That it is reliable is indicated by the fact that the ratio of free to total cholesterol obtained by its use agrees well with the ratio reported by other methods.

Total Cholesterol

The determination of the total cholesterol of plasma has been based on the method of Sackett (1925). 0.2 ml. of plasma is pipetted drop by drop in a centrifuge tube containing a mixture of 9.0 ml. of absolute alcohol and 3.0 ml. of ether. The tube is closed by a dry stopper, is shaken vigorously for about one minute and is then allowed to lie horizontally with an even distribution of the/

precipitate along the tube for 30 minutes. The mixture is then centrifuged and the supernatant liquid poured as completely as possible into a small beaker. This is placed on a hot plate and the contents carefully evaporated to dryness. The residue is extracted with 1 cc. chloroform - four times into a 10 ml. measuring cylinder and the volume made to 5 ml. with chloroform. To the 5 ml. extract, 2 ml. of acetic anhydride and 0.1 ml. of concentrated sulphuric acid are added. The cylinder is stoppered and its contents mixed and after standing in a dark place for 10 minutes, the colour is then read in a Spekker absorptiometer against water. The cholesterol equivalent is obtained from a calibration curve prepared for the purpose.

Free Cholesterol

Free cholesterol was determined by a slightly modified form of Clark's adaptation (1945) of the digitonin precipitation method of Sperry (1934). Extraction of plasma; for each 1.0 ml. of plasma about 20 ml. of acetone-alcohol (1:1) are placed in a 25 ml. volumetric flask and the plasma pipetted into this drop by drop with constant shaking. Additional acetone-alcohol is added to just below the calibration mark on each flask and extraction allowed to take place overnight at room/

temperature. The unfiltered extracts are quite stable and may be kept for some days if necessary. After extraction, the flasks are made up to volume and contents well mixed and filtered through lipid-free filter paper. Evaporation is avoided by keeping the funnels covered and working rapidly.

Precipitation of Free Cholesterol. Of each filtrate 10 ml. are pipetted into 30 ml. conical centrifuge tubes, 4 ml. of the digitonin solution (0.4 g. digitonin in 100 ml. of a 50% (V/V) solution of ethyl alcohol in water) added to each and the mixture stirred well. The rods are left in the tubes which are placed in one or more covered Mason Jars containing a layer of sand and left overnight at room temperature. The rods are then removed to a numbered rack after both the rods and the walls of the tubes have been washed down with acetone to free the precipitates. Care must be taken to return each rod to the correct tube in order to avoid the loss of any precipitate which may have adhered to it. The tubes are then centrifuged for 30 minutes at high speed, after which the supernatant fluid is poured off carefully. Centrifuging in this way allows the supernatant fluid to be poured off without disturbing the precipitate and avoids the suction method used by Clarke. At this stage the tubes may, if necessary, be left for some time/

if kept covered in Mason Jars. The stirring rods are replaced in the tubes, the rods and the tube walls are washed with 4 ml. of acetone-ether (1:2). The ether used must be free from peroxide. After thorough stirring the rods are removed and washed down with a small amount of acetone-ether. The tubes are again centrifuged at high speed for ten minutes, the supernatant fluid is removed as before, and this washing is repeated two or more times now, however, with pure ether (peroxide free). After the final washing, tubes are kept horizontally at room temperature for half an hour, incubation at 40°C as described by Clarke having been found to be unnecessary. When the precipitates are dry the rods are put back in to the original tubes.

Development and reading of color. The tubes are then placed in a bath (a deep sand-bath is convenient) whose temperature has been adjusted to exactly 110°C, where they remain for half an hour. To each tube 2 ml. of glacial acetic acid are added and stirred with the rod, after which the tube is replaced in the same sand-bath for one minute. The precipitate dissolves completely and Clarke's further incubation at 60°C is not necessary. The tubes are then transferred to a water bath at 25°C. While the temperature equilibrium is being attained a fresh mixture of acetic anhydride sulphuric acid/

is made up in proportions, 20 volumes of acetic anhydride to 1 volume of concentrated sulphuric acid, kept cool by ice cold water. A sufficient quantity is prepared to permit the addition of 8 cc. to each tube. This reagent is stable for one hour only.

To each tube are added 8 ml. of the acetic anhydride sulphuric acid mixture and the tubes are then replaced in the 25°C bath and kept in the dark for 25 minutes. Even during the subsequent reading in the photoelectric colorimeter care should be taken to keep away from light as much as possible.

The solution is read against water in photo-electric colorimeter and the result read from a calibration curve corrected for reagent blank.

NORMAL CONTROLS

As normal controls, blood was obtained from 50 healthy subjects (blood donors) within the age range of 20 to 60, and including both men and women, between whom there seems to be no significant difference in plasma cholesterol level (Kountz, Sonnenberg, Hofstatter and Wolff, 1945). The blood was withdrawn during the afternoon, 2 - 3 hours after a light lunch.

The mean values, ranges and standard/

TABLE I

THE PLASMA CHOLESTEROL IN NORMAL SUBJECTS

	Total Cholesterol mg. per 100 ml Plasma	Free Cholesterol mg. per 100 ml Plasma	$\frac{F}{T} \times 100$
Mean	195	52	26.5
S.D.	25.6	7.6	1.24
Max. found.	242	69	29
Min. found.	129	36	24
Range for 90% of observations.	161 - 235	42 - 61	

deviations are given in Table I. The standard deviations are not strictly accurate since the distribution curve is slightly skew. The plotted results for blood values for cholesterol have usually given a "skew" type of distribution curve (Wootton Maclean Smith and King (1950)) however, and they have been listed therefore in terms of range. 80% of the normal values given fall between the upper and lower 10% limits, and 98% between the upper and lower 1% limits. Thus 1% of normal subjects have blood cholesterol values lower than 115 mg., 9% between 115 mg. and 140 mg., 80% between 140 mg. and 215 mg., and 1% above 265 mg. per 100 ml. In clinical practice any single result falling outside the 10% limits is considered suspicious; a result which is outside the 1% limit is certainly abnormal.

The mean figure for total cholesterol agrees well with those in the literature derived from a study of 5^0 or more subjects. With this restriction Sunderman and Boerner (1949) list means from 194 to 235 mg. cholesterol per 100 ml. plasma, with a composite mean of about 200. The range in our series was smaller than some previously reported (e.g. Peters and Man (1943) record a mean of 194 with S.D. = 35.6 but a maximum individual observation of 320), although the S.D. does not differ very greatly from those reported.

The/

TABLE II

THE PLASMA CHOLESTEROL IN ACUTE CORONARY INFARCTION

	Total Cholesterol mg. per 100 ml Plasma	Free Cholesterol mg. per 100 ml Plasma	$\frac{F}{T} \times 100$
Mean	212	62	29
S.D.	39.2	13.5	3.3
Max. found.	305	117	38
Min. found.	129	40	24
Range for 90% of observations.	152- 275	42 - 92	25- 37

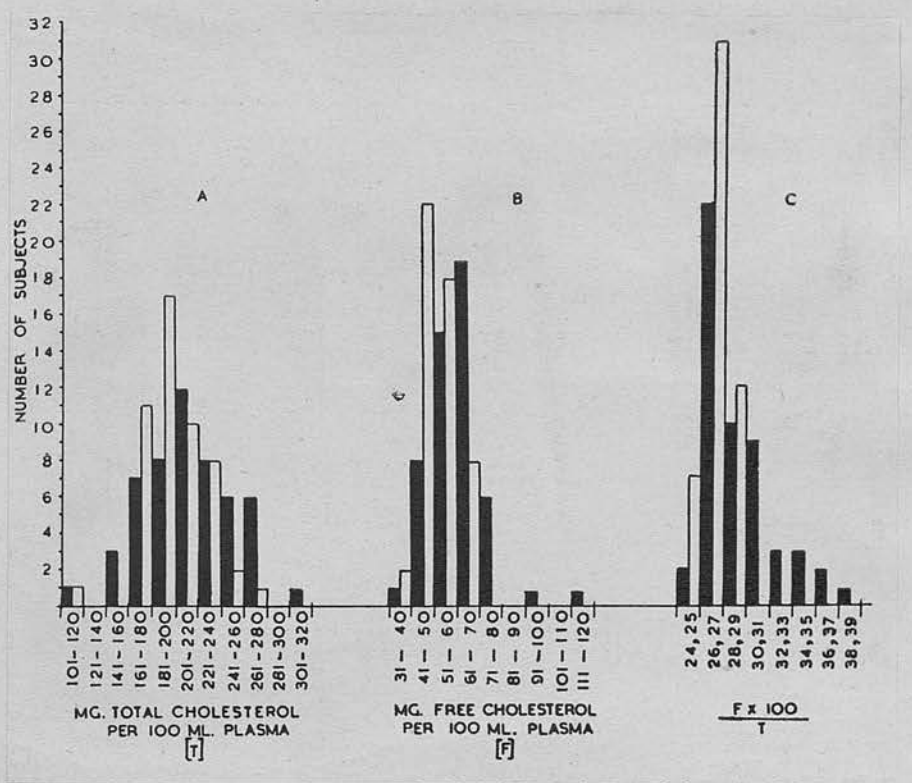
The free cholesterol concentration in the series reported here agrees well, both as regards absolute value and ratio to total cholesterol with that reported by Sperry (1936) and by Peters and Man (1943). As in their series the range of variation was very small - much less than has been reported by certain other earlier workers.

RESULTS IN RECENT CORONARY INFARCTION

The subjects were 52 patients (50 M and 2 F) aged between 42 and 72 who had been admitted to hospital because of infarction. The blood for examination was withdrawn, in every case, within 24 hours of the attack, and usually before the administration of any anticoagulant drug. The diagnosis was in all cases confirmed by clinical and electrocardiographic examination.

The data are summarised in Table II. The means and standard deviations are such as to suggest no clearly significant differences from the normal controls. Nevertheless, there is a tendency for the coronary patients to have slightly higher plasma cholesterol levels (both total and free) than the normal subjects. This tendency is shown for total cholesterol in the distribution diagram (Fig 1A) in which there is evident a distinct bunching of the coronary patients in the upper levels, and in which it is obvious that the distribution curves are skew. Examined in this/

FIGURE 1



The total plasma cholesterol (A), the free cholesterol (B), and the ratio (C) in cases of acute coronary infarction (black columns), and in healthy subjects (white columns).

way, it can be seen that only 22% of the normal subjects, 40% of the coronary infarction patients, had a total plasma cholesterol concentration over 220 mg. per 100 ml. It is still clearer in the case of the free cholesterol (Fig.1B).

The mean proportion of the total cholesterol existing in the free state is nearly the same for the two groups of subjects, but among the coronary infarction patients the range is rather greater (Fig.1C). Although 38 of the 52 patients had $\frac{F}{T} \times 100$ above the normal mean, the rather high average in this group is partly due to the inclusion in this group of six patients with $\frac{F}{T} \times 100$ above 34. In three of these functional liver damage was demonstrated by clinical examination and by the cephalin-cholesterol flocculation test; the others showed no clinical signs of hepatic disease but unfortunately tests of function were not done. One of these had suffered several previous thrombosis and had a total plasma cholesterol of 305 mg. per 100 ml. with a free cholesterol of 117 mg. per 100 ml. - the highest figure in the series; another was a chronic bronchitic of seven years standing; the third had, at the time of the coronary thrombosis, arterial occlusion at other sites.

It has already been mentioned that several American workers have noted a greater tendency to hypercholesterolaemia among the younger than among the older patients with coronary disease./

TABLE III

THE PLASMA CHOLESTEROL IN RECENT CORONARY INFARCTION - EFFECT OF AGE.

	Total Cholesterol mg.per 100 ml Plasma		Free Cholesterol mg.per 100 ml Plasma		$\frac{F}{T} \times 100$	
Age	Over 60	Under 60	Over 60	Under 60	Over 60	Under 60
Mean	199	229	57	68	28	29
Range	150-250	189-305	42-73	52-92		
Number in Group	27	25	27	25		
Number above Normal Mean.	13	21	20	18		

The results reported here support this (Table III) so far as total cholesterol is concerned. The patients over 60 years of age show no significant tendency to increased total cholesterol, but 20 out of 27 had a free cholesterol concentration in the plasma above the normal mean. The patients under 60 years of age, however, had a mean total plasma cholesterol of 229 mg/100 ml. (normal mean 195) and 21 of them, out of 25, were above the normal mean. The free cholesterol in this group was rather similar to that in the older group, although the mean value was high - possibly because it included the patients with demonstrable liver deficiency; 18 of the 25 were above the normal mean. The age difference, therefore, was not clearly demonstrated in the case of the free cholesterol.

RESULTS IN ANGINA PECTORIS WITHOUT RECENT INFARCTION.

The results from 20 patients with angina pectoris but without evidence of recent coronary infarction are summarised in Table IV.

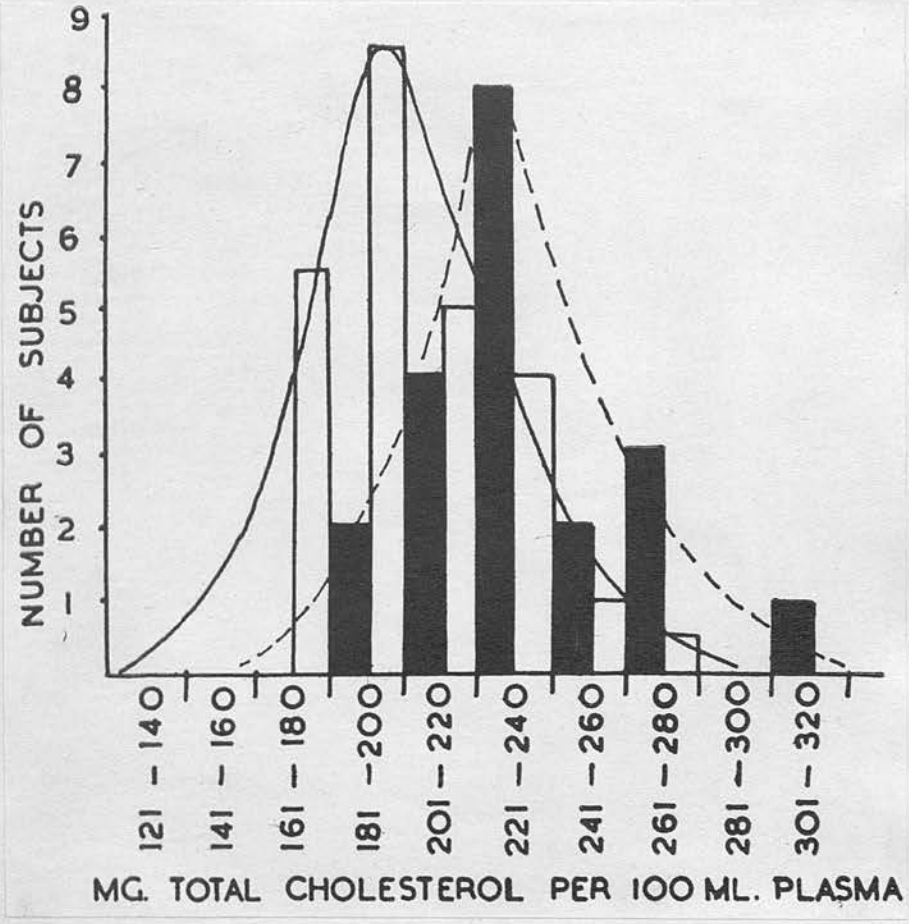
The patients were drawn mainly from those attending the Out-Patient Department. Some were known to have had acute coronary infarction some months previously but others provided no evidence of infarction. All had suffered recurrent attacks of pain over a period of several months and in/

TABLE IV

THE PLASMA CHOLESTEROL IN ANGINA PECTORIS WITHOUT RECENT INFARCTION.

	Total Cholesterol mg.per 100 ml Plasma		Free Cholesterol mg.per 100 ml Plasma		$\frac{F}{T} \times 100$	
Age	Over 60	Under 60	Over 60	Under 60	Over 60	Under 60
Mean	240	226	65.5	64	27	27
Range	204-314	185-270	57-80	46-86		
Number in Group	12	8	12	8		
Number above Normal mean.	12	7	12	6		
Mean	234.7		61.4		26	
Range (for 90% of cases)	200 - 271		54 - 80			
Total Range.	185 - 314		46 - 86			

FIGURE 2



The total plasma cholesterol in cases of angina pectoris (black columns) and in healthy subjects (white columns). For the latter, the number of individuals represented by each column is twice the number indicated on the scale.

some instances, of more than a year (four years in one instance). Those patients who had no history of acute infarction were submitted to an exercise tolerance test with electrocardiographic confirmation of the diagnosis.

It is evident that these patients showed a much greater tendency to hypercholesterolaemia than did those with acute coronary infarction. The mean value for the total plasma cholesterol was 234.7 mg. per 100 ml (normal 195) and, although many of the values were within the normal range, Fig.2 shows a clear shift of the distribution curve. Of the 20 patients in the group, 19 had plasma cholesterol in concentration greater than the normal mean.

Moreover, analysis of the fifty two cases of acute coronary infarction supports the conclusion that the occurrence of original pain is a more important factor in relation to hypercholesterolaemia than the actual coronary infarction. Of these cases, twelve gave a history of previous attacks of pain; the total plasma cholesterol ranged from 250 to 305 mg. per 100 ml., with a mean of 269.5.

The free cholesterol concentration in the plasma showed a similar general, and indeed proportional, increase above the normal; the ratio of free cholesterol to total cholesterol was/

consequently unchanged from that shown in the normal controls.

Table IV shows also that the age effect found in acute coronary infarction does not exist in the group of patients with angina pectoris but without recent infarction. There is, however, a suggestion that the tendency to hypercholesterolaemia in this group of patients is roughly related to the time which had elapsed since the first attack of anginal pain.

Since many of the patients in this group were old and all were over 50 years of age, the question of hypertension obviously arose. Among the patients with acute coronary infarction those giving a history of previous pain (i.e. those with the greatest tendency to hypercholesterolaemia) had relatively high blood pressure, and among the patients with angina but no acute infarction there was some suggestion of a rough correlation between blood pressure and the plasma total cholesterol concentration. There was, however, no clear-cut relationship, but it was obviously desirable to measure the plasma cholesterol in a group of hypertensive patients without evidence of coronary disease.

RESULTS IN HYPERTENSION WITHOUT EVIDENCE OF CORONARY DISEASE.

The plasma cholesterol concentration was/

TABLE V(a)

HYPERTENSION

	Total Cholesterol mg.per 100 ml Plasma	Free Cholesterol mg.per 100 ml Plasma	F x 100 / T
an	213	67	31
D.	50.5	17.5	
x.found.	323	122	50
n.found.	109	42	25
nge for 90% of bservations.	110-309	53-110	26-44
mber above rmal mean.	27	41	40

TABLE V(b)

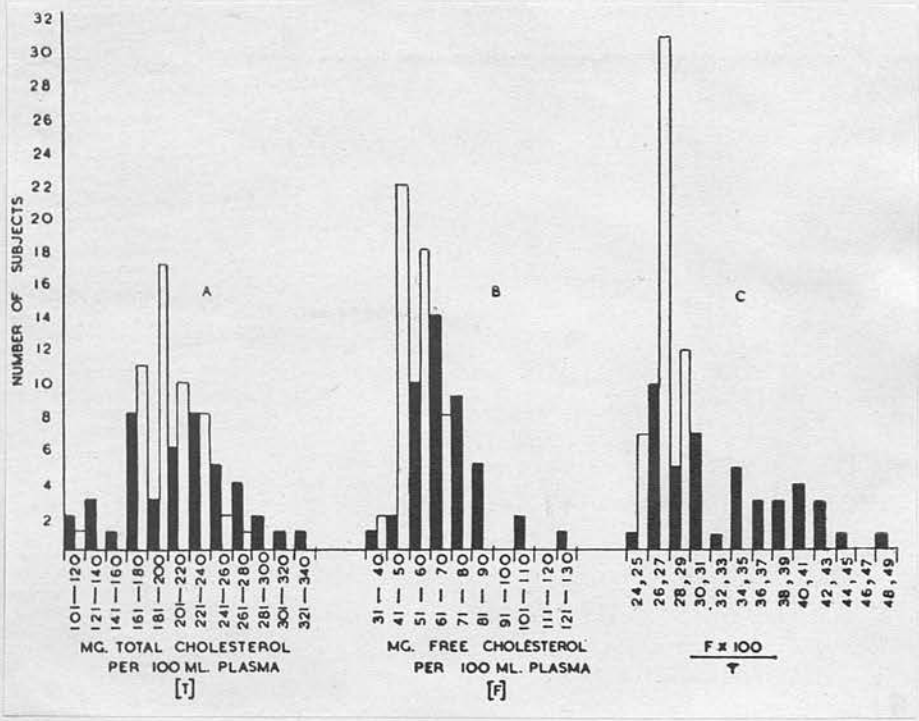
THE PLASMA CHOLESTEROL IN HYPERTENSION

	Total Cholesterol mg. per 100 ml Plasma		Free Cholesterol mg. per 100 ml Plasma		$\frac{F}{T} \times 100$	
Age	Over 60	Under 60	Over 60	Under 60	Over 60	Under 60
Mean	195	215	69	69.8	36.8	32
Range	152-266	110-309	65-80	53-110	31-44	26-44

TABLE V(c)

	Male	Female	Male	Female	Male	Female
Mean	211	214	65	73	31	34

FIGURE 3



The total plasma cholesterol (A), the free cholesterol (B), and the ratio (C) in hypertensive subjects (black columns), compared with healthy subjects (white columns).

examined in 44 hypertensive subjects, (24 men and 20 women) of all ages. Every subject had systolic blood pressure above 180 mm Hg or a diastolic pressure above 90 mm Hg (or both). The general results are summarised in Table V.

The figures for total cholesterol are very similar to those found in the patients with recent coronary infarction, though the range was rather greater and there was the same tendency for the higher figures to occur among the younger patients. There was no sex difference. The free cholesterol was distinctly higher, on the whole, than among the normal controls or even the patients with coronary disease, but the age difference appeared to have vanished. The tendency to increased free cholesterol concentrations was so marked that the ratio Free:total was considerably above the values found in the groups previously examined. This change in the ratio was actually more marked in the older patients (over 60) in contradistinction to the total hypercholesterolaemia which was more marked in the younger (below 60) patients. It is tentatively suggested that this alteration in the Free:total ratio may be ascribed to the effect of arterio-sclerolic changes on the functional efficiency of the liver. No such decreased efficiency could be demonstrated by the cephalin-cholesterol flocculation test, except in two cases, with $\frac{F}{T} \times 100$ of 35/

and 50 respectively, but this does not, of course, suffice to negative the suggestion. It is worth mentioning in this connection that McMichael (1950) has, in one post mortem case, demonstrated pathological changes in the liver of a severely hypertensive patient who showed old healed pyelonephritis and belief has been expressed that such changes are commonly associated with hypertension.

The discrepancy between the total cholesterol-age relationship and the ratio-age relationship suggested that duration of hypertension rather than age might be the determining factor, and the figures were re-examined accordingly. When the patients were classified in this way, taking 5 years duration of hypertension as the standard, the results were as shown in Table VI. It appears that the tendency to a total hypercholesterolaemia is rather greater among the patients in whom the hypertension is of relatively short duration, but it must be emphasised that this tendency has not been shown to be statistically significant. With the same caveat, though with rather more assurance, it can be suggested that the concentration of free cholesterol, and, even more markedly, the free:total ratio is more abnormal in the long duration group of patients. The progressive change of the ratio with increasing duration of hypertension is shown even more clearly in Table VIIa. The abnormality/

TABLE VI

HYPERTENSION

	Total Cholesterol mg. per 100 ml Plasma		Free Cholesterol mg. per 100 ml Plasma		$\frac{F}{T} \times 100$	
	Over 5 years duration	Under 5 years duration.	Over 5 years duration	Under 5 years duration	Over 5 years duration	Under 5 years duration
n	204	228	78	65	38	28
ge	110-300	109-323	53-122	38-88	32-50	25-35
ber in roup	20	24	20	24	20	24
ber above mal mean	7	20	20	21	20	20

TABLE VII

Duration of Hypertension in years	No. of Cases	F/T x 100 Mean	Range
0 - 2	13	28	25 - 31
2 - 4	11	30.5	26 - 37
5 - 10	10	36.8	32 - 40
10 - 15	10	41.0	37 - 50
Age in years			
40	8	27.5	26 - 29
41 - 50	12	32	26 - 37
51 - 60	18	35.5	25 - 43
61 - 70	5	38	30 - 50
70	1	31	31

of the free:total ratio is progressive, only is very much greater among the patients with hypertension of over 5 years duration, and it should be noted that this coincides with electrocardiographic evidence of progressive left ventricular hypertrophy. Naturally, the longer duration patients tend to be found among the older ones but a similar classification on the basis of age by decades (Table VIIb) showed a much less clear progression in the free:total cholesterol ratio, the ranges overlapping much more completely although the means increased. It is justifiable to conclude that, although age and duration of hypertension are not completely independent, the latter is the more important and possibly the fundamental factor.

Since some degree of renal inefficiency is almost inseparable from hypertension of long duration, it was necessary to find whether the abnormalities in the plasma cholesterol were associated with this rather than with the mere elevation of the blood pressure. In most of the cases, renal function was tested by the urea range test. Only two gave a completely normal range (i.e. 3.5% urea to below 0.5% urea) and it was therefore necessary to choose arbitrarily some lower standard which could be taken as representing a "considerable" degree of functional impairment; this standard was fixed /

TABLE VIII (a)

HYPERTENSION WITH IMPAIRED KIDNEY FUNCTION TESTS

	Total Cholesterol mg.per 100 ml. Plasma	Free Cholesterol mg.per 100 ml Plasma	$\frac{F}{T} \times 100$
Mean	217	71	32
S.D.			
Max.found.	300	122	44
Min.found.	136	53	26
Range for 90% of observations.	152-285	57-110	27-42

TABLE VIII(b)

HYPERTENSION WITH IMPAIRED KIDNEY FUNCTION TESTS

	Total Cholesterol mg.per 100 ml Plasma		Free Cholesterol mg.per 100 ml Plasma		$\frac{F}{T} \times 100$	
	Over 5 years duration	Under 5 years duration	Over 5 years duration	Under 5 years duration	Over 5 years duration	Under 5 years duration
Mean	207	192	79.8	68	38	28
Range	136-300	209-257	53-122	57-80	34-44	26-31
Number in Group.	12	6	12	6	12	6

at 2.5% urea, those failing to concentrate to this level being regarded as showing impairment of renal function. Eighteen patients fell in this group, and the figures for plasma cholesterol (Fig.VIII) are practically indistinguishable for those of the whole hypertensive group shown in Fig.VII. Evidently, impairment of renal functional efficiency is not, in these patients a factor in the abnormality of cholesterol metabolism which leads to a very slight tendency to hypercholesterolaemia combined with a much more definite increase in the proportion of the total cholesterol which remains in the free state.

RESULTS IN CONGESTIVE HEART FAILURE

The consideration that the tendency to increase in $\frac{F}{T} \times 100$ in the cases discussed in the preceding sections of this paper might be explained by some degree of liver functional efficiency due to interference with the circulation with consequent diminution of the supply of metabolites (including oxygen) to the cells led to the examination of patients with congestive heart failure. In these there is a similar disturbance which could well lead to a similar apparent diminution in liver functional efficiency and indeed the condition of "cardiac cirrhosis" has been described as a late phenomenon in recurrent congestive heart failure.

The/

TABLE IX

CONGESTIVE HEART FAILURE.

Case No	Number of Attacks	Etiology	Liver Enlargement		Ascitis	Spleen	Acute Phase			Complete Recovery Phase			Cephalin-Cholesterol Test
			During illness	After recovery			Total Cholesterol mg.per 100 ml Plasma	Free Cholesterol mg.per 100 ml Plasma	F x 100 / T	Total Cholesterol mg.per 100 ml Plasma	Free Cholesterol mg.per 100 ml Plasma	F x 100 / T	
1	One	Rheumatic	++	-ve	-ve	-ve	160	55	34	188	50	26	-ve
2	Two	"	+++	-ve	+	-ve	155	70	46	209	57	27	-ve
3	One	"	++	-ve	-ve	-ve	142	50	35	216	59	27	-ve
4	Two	"	++	-ve	-ve	-ve	129	46	35	200	57	28	-ve
5	One	"	+	-ve	-ve	-ve	257	80	31	Not examined but very early failure case.			Nil
6	Two	"	+++		-ve	-ve	150	70	46	Not examined			
7	Two	"	++		-ve	-ve	147	58	39	Not examined			
8	Multiple	"	+++	+	-ve	-ve	140	57	43	170	67	39	+ve
9	Multiple	"	+++	+	+	+	146	60	42	155	55	35	-ve
10	Multiple	Hypertensive	+++	+	+	-ve	136	57	42	150	56	37	-ve
11	Multiple	"	+++	+	+	+	164	73	44	184	70	38	+ve
12	Multiple	"	+++	+	-ve	-ve	161	65	40	185	65	35	+ve
13	Multiple	"	+++	+	-ve	-ve	160	80	50	170	70	41	+ve

The relevant data from thirteen cases are summarised in Table IX. There is an obvious diminution in the total plasma cholesterol during the acute phase of congestive failure and, since the free plasma cholesterol is not similarly reduced, the abnormality consists in a reduction of the combined cholesterol. This, of course, gives an apparent increase in $\frac{F}{T} \times 100$, such as is commonly found associated with liver disease. The clinical examination of the patients during this phase showed a rough correlation between the degree of liver enlargement and the abnormality of $\frac{F}{T} \times 100$, but none between this abnormality and the number of previous attacks.

After clinical recovery the same patients were re-examined. Those who had had not more than two previous attacks of congestive heart failure showed an increase in the total plasma cholesterol to the normal level with a return of $\frac{F}{T} \times 100$ to normal. In these patients the liver had returned to normal size and the cephalin-cholesterol test gave a negative result. The patients with several previous episodes showed a much smaller increase in the total plasma cholesterol, a continuing abnormal value for $\frac{F}{T} \times 100$, residual liver enlargement and, in most cases, flocculation in the cephalin-cholesterol test.

These results strongly suggest that the/

circulatory failure in these patients produces impairment of the hepatic functional efficiency and that long continuation or repetition of the condition leads ultimately to cellular damage. It cannot be concluded definitely that the similar derangement of the plasma cholesterol associated with hypertension and with coronary disease is also due to liver inefficiency. Since, however, these other conditions involve vascular changes which must be expected to affect the liver in the same way as does congestive heart failure, the conclusion is at least probable.

THE EFFECT OF HEPARIN ON THE PLASMA CHOLESTEROL

The existence of some connection between heparin and blood lipoids was suggested by the work of Chargaff et al (1941) and of Hahn (1943). The former workers demonstrated that heparin could rupture the bond uniting globulin with lipoid material in "lipoproteins", itself combining with the globulin. The latter observed that intravenous injection of heparin was followed, in dogs, by a rapid disappearance of alimentary lipaemia.

The importance of the plasma cholesterol concentration in relation to the concentration of other lipins at once suggested that heparin might have some influence on this, and in the course of the more general investigation of cholesterol metabolism described in the preceding pages, the opportunity arose of testing this point. This subsidiary investigation may conveniently be recorded here.

Method and results

Subjects: The subjects were nineteen patients suffering from Xanthomatosis, nephrotic syndrome, coronary infarction or pulmonary infarction.

Procedure: After a sample of venous blood had been withdrawn, heparin was given intravenously/

TABLE Xa

No.	Diagnosis.	Cholesterol Before Heparin.			Cholesterol 1st day (24 hours) after 30,000 units Heparin.			Cholesterol 2nd day (24 hours) after 10,000 units Heparin.			Cholesterol 3rd day No Heparin.			Cholesterol 4th day No Heparin.			Cholesterol 5th day No Heparin.			Cholesterol 6th day No Heparin.		
		mg. %.		F. %.	mg. %.		F. %.	mg. %.		F. %.	mg. %.		F. %.	mg. %.		F. %.	mg. %.		F. %.	mg. %.		F. %.
		T.	F.		T.	F.		T.	F.		T.	F.		T.	F.		T.	F.		T.	F.	
1*	Xanthomatosis	520	125	24	324	94	28	300	84	28	230	70	30	245	70	28	444	130	29	450	130	28
2	Nephrotic syndrome	514	130	25	300	80	26	305	80	26	300	84	24	375	100	26	425	120	27	...	...	...
3	Nephrotic syndrome	532	150	28	300	84	24	285	75	26	290	74	25	285	74	25	400	100	25	...	...	...
4	Nephrotic syndrome	323	88	27	200	60	30	205	58	28	215	62	28	220	62	28	300	80	26	...	...	...
5	Nephrotic syndrome	425	120	28	250	72	28	245	70	28	265	75	28	275	75	27	400	120	30	...	...	...

* Received tromexan throughout in addition to heparin (on the first and second days only).

TABLE Xb

No.	Diagnosis.	Heparin.	Cholesterol Before Heparin.			Cholesterol 24 hours after Heparin.			Cholesterol 48 hours after Heparin.			Cholesterol 6 weeks after Heparin.		
			Total.	Free.	%.	Total.	Free.	%.	Total.	Free.	%.	Total.	Free.	%.
6	Coronary infarction	8,000 units *statim* 6,000 units 6 hourly	245	70	28	200	55	27	220	62	28	250	67	26
7	Coronary infarction	8,000 units *statim* 6,000 units 6 hourly	230	68	29	200	53	26	210	55	26	238	68	28
8	Coronary infarction	10,000 units *statim* 6,000 units 6 hourly	220	60	27	189	60	31	200	56	28	216	62	28
9	Coronary infarction	8,000 units *statim* 6,000 units 6 hourly	230	68	29	202	60	29	200	55	27	235	66	28
10	Coronary infarction	10,000 units *statim* 10,000 units 4 hourly	250	67	26	190	56	29	210	55	26	257	67	26
11	Coronary infarction	10,000 units *statim* 10,000 units 4 hourly	200	55	27	170	48	29	175	48	27	209	57	27
12	Coronary infarction	10,000 units *statim* 10,000 units 4 hourly	211	53	25	190	54	28	192	54	28	216	59	27
13	Coronary infarction	10,000 units *statim* 10,000 units 4 hourly	200	75	37	176	66	37	...	...	...	220	76	35
14	Coronary infarction	10,000 units *statim* 10,000 units 6 hourly	268	70	26	250	70	28	255	70	27	275	80	29
15	Coronary infarction	8,000 units *statim* 6,000 units 6 hourly	235	85	36	205	70	34	210	70	33	230	80	35
16	Coronary infarction	8,000 units *statim* 6,000 units 6 hourly	230	70	29	202	60	29	...	...	...	235	68	29
17	Coronary infarction	10,000 units *statim* 10,000 units 6 hourly	255	65	25	204	52	25	...	...	...	260	74	28

TABLE Xc

No.	Diagnosis.	Cholesterol Before Heparin.			Cholesterol 24 hours after Heparin.				Cholesterol 48 hours after Heparin.			Before Discharge 6 weeks after Heparin.		
		Total.	Free.	%.	Dose.	Total.	Free.	%.	Total.	Free.	%.	Total.	Free.	%.
18	Pulmonary infarction	255	70	27	10,000 units *statim* 10,000 units 6 hourly	210	55	26	215	60	27	257	67	26
19	Pulmonary infarction	250	67	26	10,000 units *statim* 10,000 units 6 hourly	208	55	26	218	60	27	245	70	28

TABLE Xd

Tromexan Only

No.	Diagnosis.	Cholesterol Before Tromexan.			Cholesterol 24 hours after Tromexan.				Cholesterol 48 hours after Tromexan.			Cholesterol 72 hours after Tromexan.			Cholesterol 96 hours after Tromexan.		
		T.	F.	%.	Dose.	T.	F.	%.	T.	F.	%.	T.	F.	%.	T.	F.	%.
1	Coronary infarction	180	54	30	Tromexan 1·2 gm. Next day 0·9 gm.	187	57	30	190	58	30	185	57	30	187	55	29

in therapeutic doses, and further samples of blood were withdrawn at the same time each day for five days. Free and total cholesterol were determined in the plasma separated from each sample. In 14 cases a further sample of plasma was analysed after an interval of six weeks, and in the remaining five the daily analysis was continued to the fifth or sixth day.

Results: The results are summarised in Table X which shows that in every instance the administration of heparin was followed by a prompt fall in the total concentration of cholesterol in the plasma and a proportionate fall in the concentrates of free cholesterol. These falls were greater in those patients with marked hypercholesterolaemia.

Five patients, one with xanthomatosis, the others cases of nephrotic syndrome, had initial plasma total cholesterol concentrations ranging from 323 to 532 mg. per 100 ml. plasma (the normal range determined in this laboratory is 195 ± 25), and these fell by 120 to 232 mg. in 24 hrs. during which patients had each received 30,000 units of heparin given in divided doses (12,000 units initially followed by 6,000 units at six hour intervals). The free cholesterol concentrations, initially absolutely high but forming the normal proportion of the total, fell similarly so that the ratio free;total cholesterol was unaltered.

During the second 24 hour period, in spite of/

further administration of 10,000 units of heparin (one dose) there was no further decrease in the plasma concentration of free or total cholesterol. During the third 24 hour period, with no further administration of heparin the total cholesterol concentration remained unaltered but thereafter there was a rapid rise towards the initial level, which in two cases had been practically reattained on the fifth day. The concentrations of free cholesterol behaved similarly except that the restoration of the initial concentration was more nearly complete on the 5th day.

The fourteen patients with coronary or pulmonary infarction had received no heparin prior to the observations reported in Table Xb and Xc and had just been admitted to hospital with a fresh coronary thrombosis or pulmonary embolism. The initial total cholesterol concentration ranged from 200 to 268 mg. per 100 ml - i.e. in or slightly above the upper half of the normal range, and free cholesterol formed the normal percentage of the total. Each of these patients received heparin in divided doses for 24 to 48 hours. Twenty four hours after the last dose of heparin, the total cholesterol had fallen by amounts, ranging from 21 to 60 mg. per 100 ml. plasma, which were not proportionate either to the heparin dosage or to the initial level. The free cholesterol/

values fell proportionately. For therapeutic purposes, administration of heparin was followed by treatment with tromexon, but this had, of course, no effect on the figures just quoted. Forty eight hours after cessation of the heparin treatment, and 24 hours after the first dose of tromexon the concentration of cholesterol in the plasma were substantially unaltered. Daily determinations of the plasma cholesterol then ceased but six weeks later the concentration had, in all cases, returned to the initial level.

These results showed the profound influence of heparin on the plasma cholesterol concentration but did not indicate whether tromexon had a similar effect. The apparent absence of effect of tromexon in the cases quoted in Tables Xb and Xc is not conclusive, because as is shown in Table Xa the fall in plasma cholesterol produced during 24 hours administration of heparin was not increased when the drug was given for a second period of 24 hrs.

Other cases, however, show quite clearly that tromexon does not lower the plasma cholesterol level. Thus, in case I (Table Xa) the plasma cholesterol concentration rose towards the normal level at the same time as in the other cases of this group although it alone was receiving tromexon. Further, during continued administration/

of tromexon for six more days, the plasma cholesterol was maintained at 450 mg. per 100 ml. More clear cut, however, is the evidence from a patient, suffering from coronary thrombosis, who received tromexon therapeutically for about three weeks, but was at no time given heparin; the plasma cholesterol did not fall below the initial level of 190 mg. per 100 ml. on the second day of tromexon nor on any of three other occasions. (Table Xd)

Conclusions: It is evident that heparin is capable of lowering markedly the concentration of cholesterol in the plasma, that this effect is produced equally in free and esterified cholesterol, that it is greater when hypercholesterolaemia exists than when the cholesterol level is normal, and that when the lowering has been produced continued administration of heparin has no further effect. The other anticoagulant tested, tromexon, had no such action.

The twelve patients in these series who were suffering from coronary thrombosis had plasma cholesterol concentrations which were not significantly above the normal range, although all were above the normal average. The free cholesterol formed the normal proportion of the total. Similar figures have been obtained by the author in some 50 other cases reported earlier in this thesis. /

This observation, in agreement with that of Hall, Morrison and Cheyney (1948), does not support the idea that hypercholesterolaemia is the cause of coronary atheroma. Nevertheless, the metabolism of cholesterol may well undergo profound alteration without marked change in the plasma concentration and the bulk of evidence relating the occurrence of atheroma with abnormal cholesterol metabolism is too great to be ignored. It is tempting, though highly speculative, to relate the stability of cholesterol in the plasma, the concentration of heparin or similar amino-sugar esters, and the mast cells which many workers believe to be concerned in the production of such substances. Much indirect evidence indeed, can be adduced to lend plausibility to such a speculation, but until direct evidence is found, it is wiser to be content with drawing attention to the possible relation with all that it implies.

DISCUSSION

This suggestion that vascular degenerative changes slowly produce some abnormality of cholesterol metabolism is strengthened by the finding that in angina pectoris the tendency to hypercholesterolaemia is roughly proportional to the period over which anginal pains have been observed, and that, in the same subjects there is a rough parallelism between the plasma cholesterol and the blood pressure. It seems that the level of the plasma cholesterol and the degree of hypertension alike may be governed by the extent of the degenerative lesions in the blood vessels, which is a function of duration. On this basis, an acute coronary infarction may occur without widespread degenerative arterial changes, and consequently with little elevation of the plasma cholesterol. This is not to deny the proposition that the atheromatous lesion involves the deposition of cholesterol or cholesterol esters derived from the plasma; it is in agreement with the idea that there is some inherent or at least primary fault in the sub-intimal tissues; but it suggests that given this primary fault, the development of the lesion may begin without pre-existing hypercholesterolaemia to which, however, it may lead in the course of time. This/

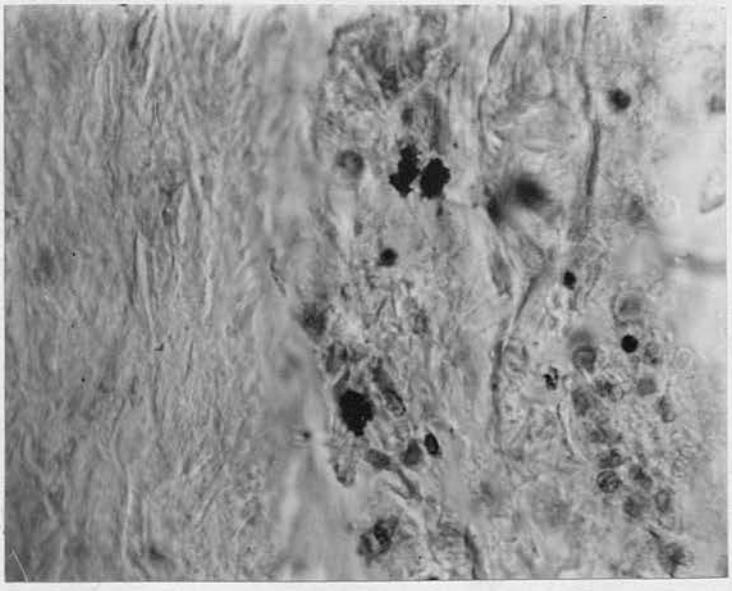
hypothesis of the sequence of events is equally consistent with the frequent occurrence of atheromatous changes in diabetes with hypercholesterolaemia and in nephrosclerosis without constant hypercholesterolaemia. The suggestion that hypercholesterolaemia is the sequel to, rather than the cause of, atheromatous changes does not oppose, but is indeed supported by the distribution of these changes in the vascular system, a distribution which led Anrep, Davis, Volhard (1931), Gregg (1934), Johnson and Dipalma (1939), Moschkowitz (1942) and others to develop the theory that atheromatous change is the result of excessive localised intravascular pressure. It was observed by Anrep, Davis and Volhard (1931), Gregg (1934) and Johnson Dipalma (1939) that the left coronary artery suffers a high hydrostatic pressure; Gregg (1940) noted that because of the smaller intramural pressure in the right ventricle than the left, the right coronary artery suffers a smaller hydro-static pressure than the left. Moschkowitz (1942) has confirmed pathologically that those arteries which support a high intravascular pressure are the most susceptible to atheromatous change.

The actual production of thrombi may be attributed to some abnormality of the clotting mechanism which is potentiated by the local injury to the artery wall (although it is not clear whether such/

clotting abnormality must necessarily be postulated). It is fairly well established (Wright 1948), Downe (1940), Jorpes (1939) that the mast cells of the liver and the smaller blood vessels produce heparin. Others (Jorpes, Holmgren and Wilander (1937), Levene, Lopezsuarez (1918) have extracted a heparin like substance from the wall of the aorta and Faber (1949) has demonstrated that deposits of cholesterol are found only where ethanol resistant metachromasia are present. He (1946) has further observed that the depletion of cholesterol is greater in these sites in hypertensive patients, though without marked increase in plasma cholesterol. Although the observations are not yet sufficiently complete for detailed publication, the writer has found mast cells in the walls of the aorta and coronary artery of normal human subjects, but in those of subjects who had suffered from atheromatous changes and it seems appropriate to interpolate here a very brief account of these observations.

As mast cells degenerate very quickly it is very difficult to obtain human tissues in a state suitable for their detection. The three cases of coronary disease studied for mast cells were obtained from patients who died accidentally and on whom post mortem/

FIGURE 4



Normal Human Aorta stained with Toluidine Blue - showing normal mast cells.

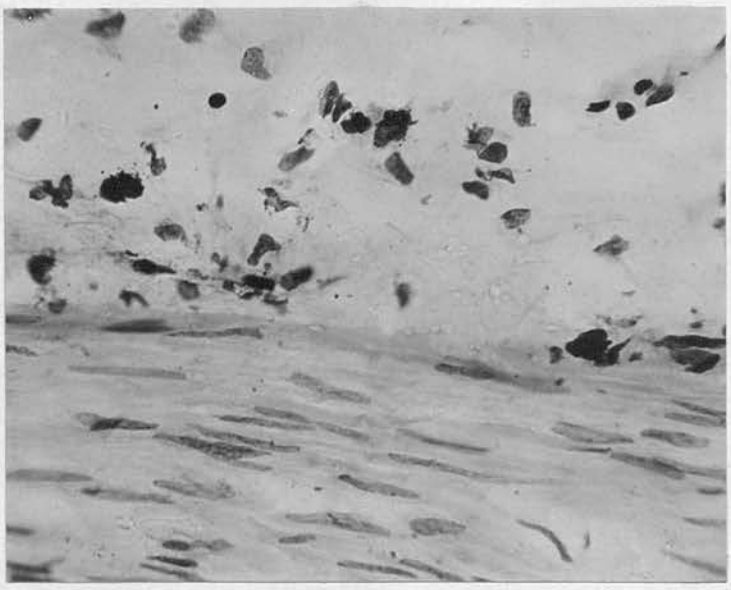
FIGURE 5

Normal human Aorta stained with Sudan Black - showing mast cells.

FIGURE 6

Normal human Coronary Artery stained with Sudan Black - showing mast cells.

FIGURE 7



Atheromatous human Coronary Artery stained with Toluidine Blue - showing less number of mast cells and rupture of mast cells, granules coming out of cells.

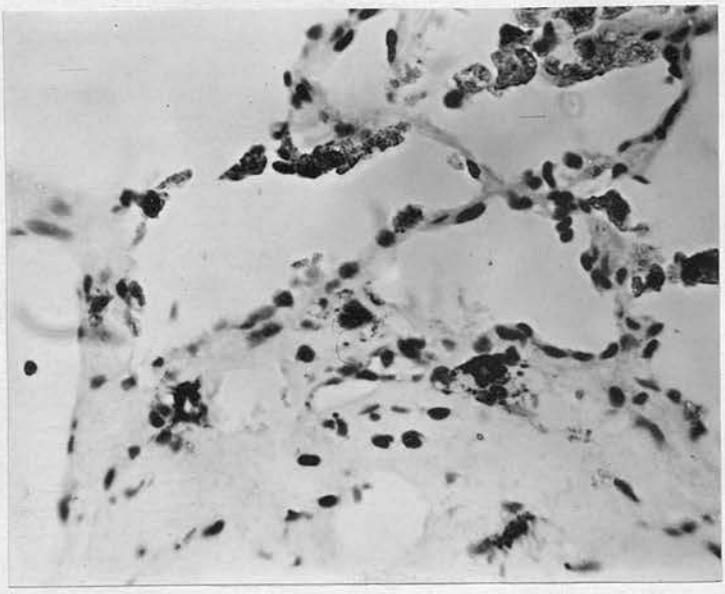
FIGURE 8

Atheromatous human Aorta stained with Toluidine Blue - showing less number of mast cells and rupture of mast cells, and abundance of granules coming out of cells.

examination was done within 5 hours of death. The tissues were fixed in 80% alcohol for preservation of mast cells, and frozen sections were later stained with Toluidine Blue which is claimed to be specific for mast cells (Fig.4). Other tissues were fixed in formalin and then frozen sections were dipped in warm alcohol or ether-acetone mixture to dissolve the lipid. It was observed by Wislocki, Bunting, Demsey (1947) that Sudanophilic granules of mast cells fixed in formalin are not dissolved by ether-acetone or alcohol. In sections placed in these solvents from 1-5 hours and then stained with Sudan black, the fatty deposits stained a clear black. Figures 5, 6 show clearly the presence of mast cells in the walls of the aorta and coronary artery of the normal subject but not in these sites in the three atheromatous subjects. In other two atheromatous subjects the mast cells though not completely absent, had undergone great degeneration (Figs.7 and 8).

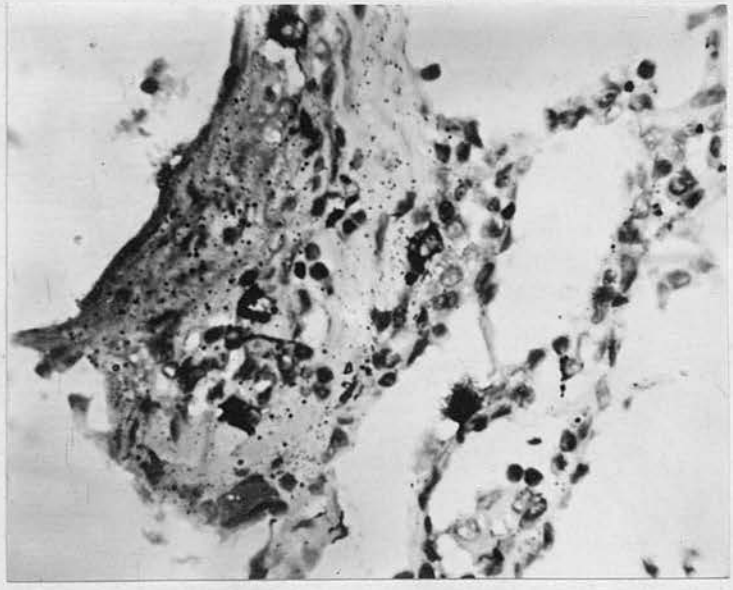
One section of lung obtained from a case of pulmonary hypertension and another section from a case suffering from pulmonary tuberculosis but with no atheromatous change were stained with Toluidine Blue. The section from the former case showed very few mast cells in comparison with that from the simple/

FIGURE 9



Section of human Lung from patient of pulmonary Hypertension stained with Toluidine Blue - showing a few mast cells.

FIGURE 10



Section of human Lung from a case of Pulmonary T.B. stained with Toluidine Blue - showing abundance of mast cells.

tuberculosis case in which a large number of mast cells was demonstrated (Figs.12 and 13). It is striking that hypocholesterolaemia has frequently been observed in simple tuberculosis and the author has also found it in a number of cases.

This evidence is admittedly scanty but it is undoubtedly suggestive of a close connection between mast cells, atheromatous change and cholesterol abnormalities and if with extended observations the local disappearance or degeneration of mast cells is found to be associated regularly with atheroma a causal relation may eventually be established.

Meanwhile it may be tentatively suggested that due sometimes to high stress and strain on the arterial walls as a result of hypertension and sometimes to other factors (e.g. food) there is damage to the mast cells with consequent reduction in heparin production so that the power to rupture the globulin-lipid bond fails since this appears to be one of the functions of heparin (Chargaff, 1942). A considerable gap is produced in the subintima of the vessels by the shrinkage of mast cells and because of affinity of these cells for lipids cholesterol gains entrance into the damaged mast cells. The affinity of mast cells for fat has been proved by Stenhaus, Ciaccio, Heinzman, Lombardo (1906) and Unger. The last mentioned of/

these workers, indeed, interprets mast cells as fat transport cells on the basis of experiments involving the injection of fat and soaps. Faber (1949) has also observed deposition of cholesterol in these subintimal places where ethanol-resistant metachromasia is present. These results and interpretations receive support from the observations that the mast cells disappear from sites where atheromatous changes have taken place.

Alder (1923) observed increase in number of mast cells in anaemia and carcinoma. Although the figures are not recorded in this thesis, the observation has been made, in anaemia and in carcinoma, of low plasma cholesterol levels, both total and free with a slight change in $\frac{F}{T} \times 100$, a finding in agreement with that of Bloor (1916), Bloor and Macpherson (1917), Dubin (1918), Ericson, Williams: Hummell, Lee, Macy (1937), Feigl (1919), Hodges, Sperry, Anderson (1943), Muller (1930), Muller and Heath (1933), Oser, Karr (1925). This finding is in agreement also with the view that increase in the number of mast cells produces extra heparin in the system and thus produces a lowered concentration of plasma cholesterol; it is a proved fact that in these groups of cases atheroma are rare.

It is evident from the data reported in this thesis and elsewhere that true hypercholesterolaemia is/

not found in acute coronary infarction, but nevertheless there may be a change of the cholesterol-globulin fraction. There may be possibilities of cholesterol-globulin complex alteration without much immediate change in the total cholesterol of the plasma, and, with tissue injury and the natural chemotaxis for cholesterol by subintimal tissue, there may be excessive deposition of cholesterol in the subintimal tissue as is suggested by Faber (1946,1949). It is permissible to speculate whether a deficiency of heparin, produced by mast cell distinction, may so alter the coagulability of the blood as to produce a tendency to thrombus formation at least on a favourable surface. It is permissible further to speculate whether a prolonged deficiency of heparin may be a factor in the hypercholesterolaemia which is associated with atheromatous changes of long duration; this is in agreement with the observation (Basu and Stewart, 1950) that injection of heparin produces a decrease in the plasma cholesterol concentration. But, whatever the mechanism, our observations indicate that the increase in the plasma cholesterol concentration follows the atheromatous changes (being, therefore an effect and not a cause) and that this increase does not involve a defect in the cholesterol esterifying mechanism.

In hypertension the position may be a little/

differemt.. Again, duration is the important factor. The mechanism, associated with vascular change, may slowly tend to an increase in the total plasma cholesterol, but this tendency is less marked than is found in the patients with anginal pains and atheromatous rather than arteriosclerotic changes. The hypertensive patients whose abnormality is of long duration show rather an increase in the free cholesterol with a consequent rise in the value of $\frac{F}{T} \times 100$ which is of the same order as is found consistently associated with hepatic functional deficiency. Although few hypertensive patients have any such deficiency demonstrable by other function tests, it is noteworthy that some degree of fatty infiltration of the liver is a common post-mortem finding in such patients. Moreover,a similar increase in $\frac{F}{T} \times 100$ has been found in cases of congestive heart failure (Hawkins, 1934, Cantarrow, 1935, Epstein, 1936) in which as we have found, when repeated failures may lead to a deficiency demonstrable in other ways - cardiac cirrhosis. There is a strong suggestion that the abnormality of the plasma cholesterol in hypertension, whilst in part due to the causes which produce vascular degeneration, are largely the result of "hypertensive liver deficiency" - a supply lack in the earlier stages which as in congestive heart/

failure may lead ultimately to actual cellular damage.

It is, however, clear that on the evidence presented the abnormalities of the plasma cholesterol in angina pectoris, in hypertension and in congestive heart failure cannot all be ascribed solely to the effect of hepatic deficiency. For whereas in the first two of these conditions there is a slight tendency for the total cholesterol to be raised and a more marked tendency for the free cholesterol to be increased with a consequential change in the ratio, congestive heart failure is characterised by a decrease in the total cholesterol with no change in the free cholesterol. It is the disproportionate change in the free and total cholesterol concentrations with an increase in F x 100/T which in all these conditions suggests hepatic functional impairment as a common factor. This, however, seems to be superimposed on other effects which may be due to diverse causes. It is possible that in congestive heart failure the hepatic damage may have progressed so far as the result of markedly defective blood supply that the liver can no longer synthesise cholesterol adequately whereas minor degrees of damage (such as can, at most, exist in the other cases studied) affect rather the esterifying and possibly the excretory powers. On the evidence available, however, it is more reasonable to suppose/

that the atheromatous changes in the blood vessels themselves produce conditions which tend to disturb the normal plasma cholesterol distribution and that the circulatory deficiency causes impairment of the hepatic functions which lead to further abnormalities in the plasma cholesterol.

SUMMARY

1. The plasma cholesterol from 50 healthy subjects (blood donors) within the age range 20 to 60, was 195 mg% ± 25.64 and Free Cholesterol 52 mg% ± 7.59, $\frac{F}{T}$ x 100 - 26.5 ± 1.24.

2. Acute Coronary Infarction.

(a) The plasma cholesterol level has been studied in 52 acute coronary infarction cases. The total cholesterol mean was 212 mg per 100 ml. ± 39.2; the free cholesterol was 62 mgms% ± 13.5; $\frac{F}{T}$ x 100 was 29 ± 3.3. Only 22% of the normal subjects, but 40% of the coronary infarction patients had a total plasma cholesterol concentration over 220 mg% per 100 ml. of plasma.

(b) The proportion of the total cholesterol existing in the free state is, on the average, nearly the same for the two groups of subjects yet 38 of the 52 coronary infarction patients had $\frac{F}{T}$ x 100 above the normal mean of 26.5 (due to inclusion in this group of six patients with $\frac{F}{T}$ x 100 above 34).

(c) The patients over 60 years of age show no significant increase of total cholesterol but 20 out of 27 had a free cholesterol concentration in the plasma above the normal mean. The patients under 60 years of age had a mean total plasma/

cholesterol of 229 mg. per 100 ml. (normal mean 195) and 21 of them out of 25 were above the normal mean. The free cholesterol was rather similar to that in older group.

(d) In 12 patients who had been suffering from previous pain, the total plasma cholesterol ranged from 250 - 305 mg% with a mean 209.5.

(e) There is thus some slight tendency in acute coronary infarction for the total plasma cholesterol to be increased, a tendency which is greater in the younger patients. The free cholesterol is much more regularly increased but the increase is not great and is independent of age.

3. Angina pectoris without recent infarction.

(a) 20 patients were studied in this group. The total cholesterol mean was 234.7 mg%; Free Cholesterol mean was 61.4; $\frac{F}{T} \times 100$ was 26.

(b) These patients showed a much greater tendency to hypercholesterolaemia; of the 20 patients 19 showed plasma cholesterol greater than normal mean.

(c) Free cholesterol concentration in the plasma showed a similar general and indeed proportional increase above the normal mean, the value of/

$\frac{F}{T}$ x 100 remaining unchanged.

(d) The age effect found in acute coronary infarction did not exist in this group of patients.

(e) The tendency to hypercholesterolaemia is roughly related to the time elapsed since the first attack of pain.

4. Hypertension without evidence of Coronary Disease.

(a) The plasma cholesterol concentration was examined in 44 hypertensive subjects, of all ages. The total cholesterol was 213.mg% $\pm$ 50.5; Free Cholesterol was 67 $\pm$ 17.5; $\frac{F}{T}$ x 100 was 31.

(b) The figures for total cholesterol are very similar to those found in the patients with recent coronary infarction but the free cholesterol was distinctly higher on the whole, and the ratio of Free:Total was consequently considerably above the normal mean and showed an average of $\frac{F}{T}$ x 100 = 31.

(c) The abnormality of the Free:Total ratio is progressive and is very much greater among the patients with hypertension of over 5 years duration. The total mean showed cholesterol for over 5 years duration. The total mean showed cholesterol for over 5 years duration of/

hypertension was 204 mg%, Free 78 mgms%, and $\frac{F}{T}$ - 38%.

(d) Duration of Hypertension rather than the age of the patient appears to be the fundamentally important factor.

5. Hypertension with impaired kidney function.

(a) 18 patients were studied in this group. The total cholesterol mean was 217 mg%; Free Cholesterol mean was 71 mgms%; $\frac{F}{T}$ x 100 was 32.

(b) The figures for plasma cholesterol are practically indistinguishable for those of the whole Hypertensive group, and it appears that renal efficiency as such is not a factor in determining the abnormality or otherwise of the plasma cholesterol level.

6. Congestive Heart Failure.

13 cases were studied in this group, during the acute illness and later after recovery.

(a) The abnormality consists in a reduction of the combined cholesterol of the plasma.

(b) There is a rough correlation between the degree of liver enlargement and abnormality of $\frac{F}{T}$ x 100.

(c) The patients with several previous episodes/

showed much smaller increase of total cholesterol during the recovery phase and in these $\frac{F}{T} \times 100$ showed a continuing abnormal value with a residual liver enlargement and flocculation with cephalin-cholesterol.

7. Injection of heparin causes a fall in the plasma cholesterol proportionately distributed between the free and combined fractions. The fall is greater when the initial plasma cholesterol level is high. Tromexan does not show this phenomenon.

8. The walls of normal human aorta and coronary artery have been shown to contain mast cells. With atheromatous changes in these sites the mast cells disappear or show degeneration.

9. On the basis of these findings it is suggested that changes in the plasma cholesterol are the result rather than the cause of vascular disease whether this effects the coronary artery in particular or not. It is further suggested that vascular degeneration by interfering with the supply of blood to the liver slowly produces functional deficiency of that organ and that the changes in the free cholesterol of the plasma must be referred at least largely to this. In vascular disease of long duration/

the liver impairment may become irreversible and may account secondarily for the changes in the total cholesterol which seem to occur later in the history of angina pectoris or hypertension. It is further suggested that a factor in the plasma cholesterol changes may be degeneration of the mast cells in the arterial walls (observed); lung (observed) and liver (not yet observed but inferred as a probable result of interference with the blood supply if not a primary phenomenon); this suggestion however is made tentatively because the direct evidence is still slight although much indirect evidence can be adduced in its support.

APPENDIX

DETAILED RESULTS SUMMARISED IN TABLES 1 - 4

TABLE I

PLASMA CHOLESTEROL CONCENTRATION IN NORMAL PERSONS

Case No	mg. Chol. per 100 ml of plasma			Case No	mg. Chol. per 100 ml. of plasma		
	FREE	TOTAL	$\frac{F}{T} \times 100$		FREE	TOTAL	$\frac{F}{T} \times 100$
1	42	161	26	28	47	166	28
2	50	190	26	29	57	223	25
3	46	180	25	30	55	190	28
4	50	185	27	31	54	192	28
5	50	204	24	32	42	161	26
6	50	196	25	33	50	190	26
7	54	192	28	34	50	186	26
8	36 *	129 *	27	35	54	200	27
9	44	176	24	36	61	223	27
10	50	186	26	37	69	242	28
11	50	181	27	38	46	166	27
12	54	200	27	39	50	196	25
13	57	209	27	40	57	209	27
14	57	214	26	41	55	190	28
15	61	228	26	42	69	235	29
16	57	212	27	43	50	185	27
17	50	176	28	44	38	132	28
18	46	166	27	45	46	178	26
19	46	166	27	46	57	214	26
20	55	190	28	47	61	228	26
21	54	205	26	48	57	212	27
22	54	202	26	49	55	210	26
23	69 **	242 **	28	50	50	181	27
24	69	238	29				
25	57	223	25				
26	61	223	27				
27	50	176	28				

Free Cholesterol (Mean = 52 mg per 100 ml
(Range for 90% of observations 41 - 61
(Standard Deviation = ± 7.59

Total Cholesterol (Mean = 195 mg per 100 ml
(Range for 90% of observations 161 - 235
(Standard Deviation = ± 25.64

$\frac{F}{T} \times 100$ (Mean = 26.5
(Standard Deviation = ± 1.241

* Lowest value found
** Highest value found

TABLE 2

ACUTE CORONARY INFARCTION.

NO.	AGE	SEX	DIAGNOSIS	CHOLESTEROL mg% per 100 ml of plasma TOTAL	FREE	F% / T	E.C.G. DIAGNOSIS	LIVER	LIVER FUNCTION TEST	PAIN PREVIOUS PAIN	PAIN NO PAIN	BLOOD PRESSURE	REMARKS
1	60	M	C.T.	185	50	27	+	Nil	Not done		+	110/90	
2	62	M	C.T.	166	53	32	+	Nil	Not done		+	120/80	
3	70	M	C.T.	164	42	26	+	Nil	Not done		+	110/75	
4	64	M	C.T.	152	53	34	+	++	Cholesterol Cephalin +		+	100/80	
5	67	M	C.T.	180	46	26	+	Nil	Not done		+	120/80	
6	60	M	C.T.	216	59	27	+	Nil	Not done		+	105/80	
7	60	M	C.T.	219	57	26	+	Nil	Not done		+	110/86	
8	50	M	C.T.	269	73	27	+	Nil	Not done	+		160/90	
9	61	M	C.T.	271	73	26	+	Nil	Not done	+		140/80	
10	60	M	C.T.	250	65	26	+	Nil	Not done	+		145/95	
11	69	M	C.T.	212	57	27	+	Nil	Not done		+	120/90	

ACUTE CORONARY INFARCTION (CONTD).

NO.	AGE	SEX	DIAGNOSIS	CHOLESTEROL mg% per 100 ml of plasma TOTAL	FREE	F% / T	E.C.G. DIAGNOSIS	LIVER	LIVER FUNCTION TEST	PAIN PREVIOUS PAIN	PAIN NO PAIN	BLOOD PRESSURE ON DAY OF ADMISSION AND NEXT DAY	REMARKS
12	50	M	C.T.	204	52	25	+	Nil	Not done		+	110/95	
13	62	M	C.T.	187	57	30	+	Nil	Not done		+	110/70	
14	64	M	C.T.	190	46	24	+	Nil	Not done		+	120/88	
15	61	M	C.T.	200	53	26	+	Nil	Not done		+	118/90	
16	59	M	C.T.	166	46	27	+	Nil	Not done		+	125/70	
17	50	M	C.T.	220	60	27	+	Nil	Not done		+	110/80	
18	49	M	C.T.	235	67	29	+	Nil	Not done		+	120/96	
19	61	M	C.T.	152	40	26	+	Nil	Not done		+	100/70	
20	67	M	C.T.	167	50	29	+	Nil	Not done		+	120/95	
21	54	M	C.T.	189	60	31	+	Nil	Not done		+	120/85	

ACUTE CORONARY INFARCTION (CONTD).

NO.	AGE	SEX	DIAGNOSIS	CHOLESTEROL mg% per 100 ml of plasma TOTAL	FREE	F% T	E.C.G. DIAGNOSIS	LIVER	LIVER FUNCTION TEST	PAIN PREVIOUS PAIN	PAIN NO PAIN	BLOOD PRESSURE ON DAY OF ADMISSION AND NEXT DAY		REMARKS
22	56	M	C.T.	220	67	30	+	Nil	Not done		+	105/90		
23	58	M	C.T.	216	60	27	+	Nil	Not done		+	110/85		
24	46	M	C.T.	202	60	29	+	Nil	Not done		+	110/86		
25	68	M	C.T.	177	54	30	+	Nil	Not done		+	120/90	105/70	
26	57	M	C.T.	205	70	34	+	++	Cholesterol Cephalin ++		+	130/94		
27	68	M	C.T.	250	80	32	+	Nil	Not done	Yes		140/90		
28	68	M	C.T.	176	66	37	+	Nil	Not done		+	110/80		Arterial
29	58	M	C.T.	305	117	38	+	Nil	Not done	+		150/95		
30	42	M	C.T.	275	92	29	+	Nil	Not done	+		150/85		
31	63	M	C.T.	232	70	30	+	Nil	Not done		+	120/85		
32	49	M	C.T.	190	70	36	+	Nil	Not done		+	120/60	105/60	Chronic Bronchitis

ACUTE CORONARY INFARCTION (CONTD).

NO.	AGE	SEX	DIAGNOSIS	CHOLESTEROL mg% per 100 ml of plasma TOTAL	CHOLESTEROL mg% per 100 ml of plasma FREE	CHOLESTEROL mg% per 100 ml of plasma F% / T	E.C.G. DIAGNOSIS	LIVER	LIVER FUNCTION TEST	PAIN PREVIOUS PAIN	PAIN NO PAIN	BLOOD PRESSURE ON DAY OF ADMISSION AND NEXT DAY		REMARKS
33	63	M	C.T.	150	48	32	+	Nil	Not done		+	120/80		
34	60	M	C.T.	235	68	29	+	Nil	Not done		+	110/90		
35	61	M	C.T.	240	68	28	+	Nil	Not done		+	150/95	125/85	
36	47	M	C.T.	260	70	26	+	Nil	Not done	+		140/95		
37	72	M	C.T.	180	64	28	+	Nil	Not done		+	110/75		
38	52	M	C.T.	270	86	29	+	Nil	Not done	+		175/95	145/80	
39	61	M	C.T.	185	57	30	+	Nil	Not done		+	120/80		
40	58	M	C.T.	228	61	26	+	Nil	Not done		+	130/90		
41	52	F	C.T.	252	70	27	+	Nil	Not done		+	120/70		
42	62	M	C.T.	238	61	26	+	Nil	Not done		+	110/75		
43	63	M	C.T.	204	65	31	+	Nil	Not done		+	110/70		

ACUTE CORONARY INFARCTION (CONTD).

NO.	AGE	SEX	DIAGNOSIS	CHOLESTEROL mg% per 100 ml of plasma TOTAL	FREE	F/T %	E.C.G. DIAGNOSIS	LIVER	LIVER FUNCTION TEST	PAIN PREVIOUS PAIN	PAIN NO PAIN	BLOOD PRESSURE ON DAY OF ADMISSION AND NEXT DAY			REMARKS
44	51	M	C.T.	228	65	28	+	Nil	Not done		+	110/60			
45	58	F	C.T.	223	61	27	+	Nil	Not done		+	105/75			
46	51	M	C.T.	250	65	26	+	Nil	Not done	+		200/160	170/130	135/100	
47	55	M	C.T.	129	46	35	+	+	Cholesterol Cephalin +		+	110/95			
48	68	M	C.T.	209	57	27	+	Nil	Not done		+	112/80			
49	53	M	C.T.	257	80	31	+	Nil	Not done	Yes		160/105	145/85		
50	59	M	C.T.	209	61	28	+	Nil	Not done	No		125/70			
51	55	M	C.T.	266	80	31	+	Nil	Not done	Yes		170/100	140/80		
52	45	M	C.T.	271	73	26	+	Nil	Not done	+		135/90			
53	62	M	C.T.	204	65	31	+	Nil	Not done		+	100/75			

E.C.G. All Electrocardiographic examinations were done by standard and unipolar limb and chest leads.
C.T. = Coronary Thrombosis.

TABLE 3

ANGINA PECTORIS.

NO.	AGE	SEX	BLOOD PRESSURE	CHOLESTEROL mg% per 100 ml of plasma TOTAL	FREE	F%/T	PAIN	DURATION	E.C.G. Before Exercise	E.C.G. After Exercise
1	54	M	150/96	185	46	24	+	4 months	-ve	-ve
2	67	M	180/110	204	57	27	+	8 months	-ve	-ve
3	79	M	140/100	228	61	26	+	1 year 4 months	-ve	-ve
4	64	M	178/114	314	80	25	+	4 years	-ve	+ve
5	53	M	145/95	230	65	28	+	$2\frac{1}{2}$ years	-ve	+ve
6	56	F	140/96	220	64	29	+	1 year	-ve	-ve
7	58	F	150/96	200	54	27	+	3 years 2 months	-ve	-ve
8	66	M	160/110	235	69	29	+	6 months	+	+
9	70	M	155/105	223	61	27	+	1 year	+	+
10	74	M	185/115	242	69	28	+	9 months	+	+
11	56	M	155/96	209	57	27	+	6 months	-ve	+ve
12	68	M	160/96	216	65	30	+	2 years	-ve	+ve

ANGINA PECTORIS (CONTD).

NO.	AGE	SEX	BLOOD PRESSURE	CHOLESTEROL mg% per 100 ml of plasma TOTAL	FREE	F%/T	PAIN	DURATION	E.C.G. Before Exercise	E.C.G. After Exercise
13	70	M	180/115	271	73	26	+	3 years	+	+
14	67	F	146/96	252	69	27	+	1 year	-ve	+ve
15	56	M	140/86	231	60	25	+	9 months	-ve	-ve
16	50	M	200/120	270	86	29	+	2½ years	-ve	+ve
17	61	M	160/100	238	61	25	+	3 months	-ve	-ve
18	63	M	150/110	228	61	26	+	5 months	+	+
19	59	M	170/110	266	80	30	+	3 years	-ve	+ve
20	65	M	150/100	233	61	26	+	6 months	-ve	-ve

E.C.G. All Electrocardiographic examinations were done by standard, unipolar limb and chest leads.

(i) -ve - Nothing to suggest.

(ii) +ve - Definite finding of Angina.

(iii) + - Tests not done as these patients suffered from acute coronary infarction before.

TABLE 4

HYPERTENSION

No.	Age	Sex	Blood Pressure mm Hg	Duration of Illness	CHOLESTEROL mg% per 100 ml. of plasma Total	Free	Free/Total %	Liver	Liver Function Tests Cholesterol Cephalin	Kidney Function Tests	Electro-cardio-graphic Changes
										Urea Range	
1	21	M	180/90	6 months	240	70	29	-ve	-ve	3.6%-1016 0.4%-1001	No Abnormality
2	60	M	200/110	1 year	164	42	25	-ve	-ve	3%-1018 0.3%-1003	"
3	40	M	190/110	1 year	209	57	27	-ve	-ve	3.5%-1018 0.4%-1002	"
4	35	F	200/130	1 year	255	72	28	-ve	-ve	2.6%-1022 0.4%-1000	"
5	35	M	160/100	1 year	233	61	26	-ve	-ve	3%-1020 0.4%-1004	"
6	40	M	180/100	1 year	209	57	27	-ve	-ve	3%-1018 0.5%-1004	"
7	53	F	250/150	1 year	230	73	31	-ve	-ve	6AM -1010 7AM -1015 8AM -1005 9AM -1004	"
8	36	F	180/110	1 year	238	70	29	-ve	-ve	2.2%-1018 0.3%-1004	"

HYPERTENSION

No.	Age.	Sex	Blood Pressure mm Hg	Duration of Illness	CHOLESTEROL mg% per 100 ml. of plasma Total	Free	Free/Total %	Liver	Liver Function Tests Cholesterol Cephalin	Kidney Function Tests	Electro-cardio-graphic Changes
										Urea Range	
9	50	M	230/130	1½ years	235	69	29	-ve	-ve	2.8%-1020 0.4%-1004	No Abnormality
10	39	M	150/110	1½ years	228	61	26	-ve	-ve	2.5%-1016	"
11	52	F	210/120	1½ years	228	70	30	-ve	-ve	3%-1022 0.4%-1000	"
12	55	F	198/115	1½ years	223	61	27	-ve	-ve	3.2%-1018 0.3%-1004	"
13	50	M	210/120	2 years	269	73	27	-ve	-ve	3.6%-1020 0.4%-1002	"
14	44	M	195/110	2½ years	176	53	30	-ve	-ve		"
15	45	F	190/110	2½ years	220	60	27	-ve	-ve		"
16	79	M	200/115	3 years	204	65	31	-ve	-ve		"
17	40	M	190/110	3 years	272	80	29	-ve	-ve		"

HYPERTENSION

No.	Age.	Sex.	Blood Pressure mm Hg	Duration of Illness	CHOLESTEROL mg% per 100 ml. of plasma Total	Free	Free% Total	Liver	Liver Function Tests Cholesterol Cephalin	Kidney Function Tests	Electro-cardio-graphic Changes
18	45	M	200/120	3 years	204	65	31	-ve	-ve	Urea Range	No Abnormality
19	45	F	180/115	3½ years	188	50	26	-ve	-ve		"
20	59	M	180/115	3½ years	309	84	27	-ve	-ve		"
21	60	M	230/130	4 years	109	38	35	+ve	+ve		"
22	65	M	190/110	4 years	266	80	30	-ve	-ve		"
23	60	M	200/120	4 years	323	88	27	-ve	-ve		"
24	46	F	220/130	4 years	257	80	31	-ve	-ve	2.2%-1018 0.2%-1000	Left Ventricular Hypertrophy
25	45	M	200/120	5 years	250	90	36	-ve	-ve		" "
26	61	F	215/115	5 years	275	90	32	-ve	-ve		" "
27	47	M	200/110	6 - 7 years	192	72	37	-ve	-ve	2.3%-1018 0.4%-1000	" "

HYPERTENSION

No.	Age	Sex	Blood Pressure mm Hg	Duration of Illness	CHOLESTEROL mg% per 100 ml. of plasma Total	CHOLESTEROL mg% per 100 ml. of plasma Free	Free% Total	Liver	Liver Function Tests Cholesterol Cephalin	Kidney Function Tests Urea Range	Electro-cardio-graphic Changes
28	50	M	210/125	7 years	185	65	35	-ve	-ve	2%-1016 0.3%-1002	Left Ventricular Hypertrophy
29	60	F	230/130	8 years	161	65	40	-ve	-ve		" "
30	60	F	280/140	9 years	161	65	40	-ve	-ve		" "
31	47	F	220/110	9 years	162	55	34	-ve	-ve	3%-1018 0.4%-1004	" "
32	50	M	210/115	9 years	176	65	38	-ve	-ve	2.5%-1016	" "
33	60	F	250/135	10 years	140	56	40	-ve	-ve	3%-1020 0.4%-1004	" "
34	67	M	215/120	10 years	152	53	34	-ve	-ve	2.5%-1020 0.3%-1004	" "
35	53	F	190/120	11 years	242	90	37	-ve	-ve	2.2%-1016 0.4%-1002	" "

HYPERTENSION

No.	Age	Sex	Blood Pressure mm Hg	Duration of Illness	CHOLESTEROL mg% per 100 ml. of plasma Total	CHOLESTEROL mg% per 100 ml. of plasma Free	Free % Total	Liver	Liver Function Tests Cholesterol Cephalin	Kidney Function Tests	Electrocardiographic Changes
										Urea Range	
36	58	M	200/130	12 years	220	76	35	-ve	-ve	Inulin-Diodrast Low Value	Left Ventricular Hypertrophy
37	53	F	200/120	13 years	257	110	42	-ve	-ve	" "	" "
38	58	F	250/130	13 years	176	65	38	-ve	-ve	2%-1016 0.2%-1000	" "
39	58	F	260/130	14 years	285	110	39	-ve	-ve	2.8%-1024 0.3%-1002	" "
40	60	F	260/140	14 years	136	57	42	-ve	-ve	2.6%-1020 0.4%-1002	" "
41	54	F	234/150	15 years	300	122	40	-ve	-ve	Inulin-Diodrast Low Value	" "
42	66	F	216/135	15 years	164	72	44	-ve	-ve	" "	" "
43	62	M	270/135	15 years	140	55	50	+ve	+ve		" "
44	58	M	220/125	15 years	140	57	43	-ve	-ve		" "

All electrocardiographic examinations were done by standard, unipolar limb and chest leads

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